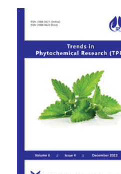
Trends in Phytochemical Research (TPR)

Journal Homepage: https://tpr.shahrood.iau.ir/



Original Research Article

Antioxidant, antimicrobial and anti-prostate cancer activit of the extracts from different parts of *Etlingera velutina* (Ridl.) R. M. Sm (Zingiberaceae)

Behnam Mahdavi*[1] And Majid Mohammadhosseini[2,3]

[1]Department of Chemistry, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran
[2]Department of Chemistry, College of Basic Sciences, Shahrood Branch, Islamic Azad University, Shahrood, Iran
[3]Nanotechnology Research Center, Islamic Azad University, South Tehran Branch, Tehran, Iran

ABSTRACT

The current report concerns with phytochemical and biological analysis of the organic extracts with different polarities, *e.g.*, methanol, ethanol, ethyl acetate and acetone from some organs of *Etlingera velutina* (Ridl.) R. M. Sm (leaves, rhizomes and stems). This study revealed remarkable antioxidant potentialities of the prepared extracts using DPPH and β-carotene bleaching (BCB) assays as well as notable antibacterial features using disc diffusion and minimum inhibitory concentration methods. More importantly, the antiproliferative of methanolic extracts of the plant parts was evaluated against human prostate adenocarcinoma cell line using MTT assay.

ARTICLE HISTORY

Received: 02 November 2022
Revised: 04 December 2022
Accepted: 31 December 2022
ePublished: 31 December 2022

KEYWORDS

Antimicrobial activity
Antioxidant activity
Antiproliferative
Etlingera velutina (Ridl.) R. M. Sm
Extract
Zingiberaceae

dor: 20.1001.1.25883623.2022.6.4.7.9

1. Introduction

The life of human beings has been closely related to a wide variety of medicinal and herbal plants from the time immemorial. In fact, these materials have remarkable therapeutic potential to address a broad spectrum of persistent diseases and are also rich sources of valuable natural products with a number of unique and vital biological, pharmaceutical and phytochemical activities (A.M. Abdel-Rahman et al., 2022; Kazeminia et al., 2022; Nalawade et al., 2022; Pardeshi et al., 2022). In fact, medicinal plants are usually regarded as an important resource for healthcare and economics from the regional to the international trading. According to the report from the WorldBank (2004), trade in medicinal plants has appraised to the value of 60 billion USD per year and is growing at a rate of 7% a year. On the other hand, natural products are defined as chemical substances that are obtained from natural origins, *e.g.*, plants, animals, and microorganisms from terrestrial or marine sources. The characterized bioactive natural products exert biological effects and therapeutic activity against human and animal diseases (Colegate and Molyneux, 2008) and have been the principal sources of drugs for human beings for thousands of years. Great cultures such as ancient Persian, Indian, Chinese, and North African have provided written evidence on man's experiences in utilizing plants for the cure of a vast variety of diseases (Phillipson, 2001). About 80% of the world's drugs are obtained from plant origin, particularly those used in tropical regions. However, many plants in these regions have not yet been identified with a specific name, and only around 15% of the plants in these regions have been considered for their pharmaceutical potential (Leland et al., 2006). Despite the fact that the use of natural products from plants in drug discovery has decreased, natural products still hold the highest priority in terms of drug source. Natural products offer greater structural variety than standard

Corresponding author: Behnam Mahdavi
Tel: +98-51-44013319 ; Fax: +98-51-44012669
E-mail address: b.mahdavi@hsu.ac.ir; behnammahdavi@yahoo.com, doi: 10.30495/tpr.2022.699180

combinational chemistry, and can be conveniently used to find new low molecular weight priorities that are efficient in a wide range of assays (Harvey, 2000). Zingiberaceae is one of the most important plant families consisting of *ca.* 50 genera and about 1600 known species many of which have remarkable therapeutic potentials and are widely used in the traditional folk medicine of many countries (Christenhusz and Byng, 2016). The main distribution areas of the plants of this family are mainly located in some parts of Asia, America as well as tropical Africa (Mozaffarian, 2003). *E. velutina* (Ridl.) R. M. Sm, as one of the species of Zingiberaceae, grows in the shade or gaps of forests and deserted gardens, often in wet habitats on lower slopes, valleys or near streams at 30-1100 m (Zargari, 1991). The flowers of *E. velutina* have a distinct white margin to the lateral lobes of labellum as shown in Fig. 1..

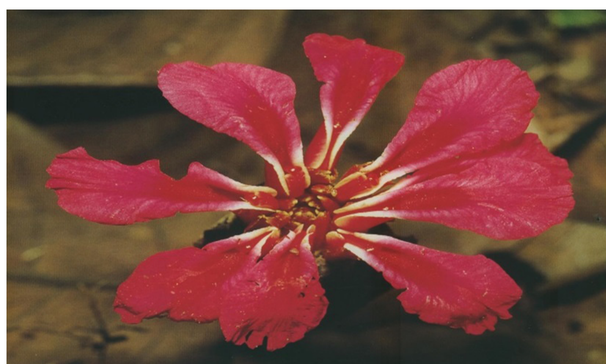


Fig. 1. Flower of *Etlingera velutina*, Source: (Poulsen, 2006).

'*Tepus kenyalang*', '*Tolidus sarou*', '*Tubu sia*', and '*Tahau*' are some of the local names for this herbal species. *E. velutina* (Ridl.) R. M. Sm is used as flavoring agent in the traditional folk medicine of many countries. In addition, the stems juice of the young of this species can be used as an efficient remedy against snakebite. The shoots of *E. velutina* (Ridl.) R. M. Sm are edible and the leaves are utilized for wrapping rice, as well (Poulsen, 2006). This report is in an endeavor for the evaluation of the antioxidant and antimicrobial activities of different organic extracts from the leaves, rhizomes and stems of *E. velutina* (Ridl.) R. M. Sm as well as the assessment of the relevant antiproliferative activities.

2. Experimental

2.1. Regions and materials

All of the chemical reagents used were of highest purity and analytical reagent grade. All the chemicals were used without further purification. The reagents used for the evaluation of antioxidant activity were all purchased from Merck. The MTT assay for the assessment of cytotoxicity (antiproliferative) activity was provided by Sigma-Aldrich. All of the Gram-positive and Gram-negative bacterial strains were obtained from the Microbiology Laboratory culture collection, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia and verified by standard microbiology method.

2.2. Plant material and extract

The studied parts involving the rhizomes (R), stems (S) and leaves (L) of *E. velutina* (Ridl.) R. M. Sm. were collected from their natural habitat in Sabah, Malaysia and subsequently dried in the shade away from direct sunlight. The dried plant parts including rhizome (100 g), stem (150 g), and leaf (150 g) were ground and extracted with different solvents like ethyl acetate (Ea), acetone (A), ethanol (Et), and methanol (M). The plant parts were macerated non-consecutively in each solvent at room temperature for 72 h. After filtration, the solvents were evaporated under reduced pressure using a Heidolph rotary evaporator (Laborota 4000 eco).

2.3. Antioxidant activity assay

The methods described below were based on the relevant procedures previously reported by Mahdavi et al. (2013) with some modification.

2.3.1. Determination of total phenolic content (TPC)

Briefly, 0.5 mL of Folin-Ciocalteu reagent (FCR, 10% in distilled water) was added to a vial containing 0.5 mL of each extract of *E. velutina* (Ridl.) R. M. Sm (1000 μg/mL in methanol) and 1.5 mL of distilled water. The resulting mixture was then vigorously shaken for 5 min. Immediately after, 2 mL of sodium carbonate solution (10 w/v%) was added, the mixture was shaken again and incubated in the dark for 2 h at room temperature. The absorbance was then measured at 760 nm using a Varian (Cary 50 conc) UV/Vis. spectrophotometer. The analyses were carried out in triplicate and the TPC was determined in as mg of gallic acid equivalent (GAE) per gram of extract (mg GAE/g extract).

2.3.2. Determination of radical scavenging activity (RSA%)

For the evaluation of the radical scavenging activity (RSA%) of the prepared extracts, an aliquot of each extract (1.5 mL) of *E. velutina* (Ridl.) R. M. Sm at 20, 100, 500, and 1000 μg/mL was first added to 1 mL of DPPH (0.1 mM) in methanol. The mixture was shaken for 1 min., and allowed to stand in the dark for 90 min. at room temperature. The absorbance of each solution was finally read at 517 nm. Ascorbic acid (AscA), butylated hydroxytoluene (BHT), gallic acid (GA), and α-tocopherol (α-Toc.) were used as positive controls. All measurements were carried out in triplicate on 3 days. The RSA was calculated according to the following equation:

$$RSA\% = [(A_c - A_s)/A_c] \times 100 \quad \text{(Eqn. 1)}$$

Where A_c and A_s respectively stand for the absorbance of the control (DPPH solution without extract), and the absorbance of the sample (extract + DPPH solution).

2.3.3. β-Carotene bleaching (BCB) assay

5 mL of β-carotene solution in chloroform (1 mg/mL) was added to a flask containing 50 μL of linoleic

acid and 500 μL of Tween 40. The chloroform was evaporated under vacuum at 45 °C for 10 min. Then, 125 mL of oxygenated water was added and the mixture was vigorously shaken to form an emulsion. This was followed by the addition of 2.5 mL of the emulsion to a 0.2 mL portion of the extract solution (1000 μg/mL in methanol) and the absorbance was immediately read at 470 nm. The mixtures incubated at 50 °C and the relevant absorbances were measured at 45 min intervals up to 180 min. All measurements were carried out in triplicate. Using the following formula, the antioxidant activity (AA) was evaluated in terms of bleaching capability of β-carotene:

$$AA\% = [1-(A^s_0 - A^s_t)/(A^c_0 - A^c_t)] \times 100 \qquad \text{(Eqn. 2)}$$

where A^s_0 and A^c_0 are absorbance of the extract and control (2.5 mL of the emulsion and 0.2 mL of methanol) at zero time, A^s_t and A^c_t are absorbance of the extract and control after 180 min.

2.3.4. Ferrous ion chelating (FIC) assay

To monitor the ferrous ion chelating ability of the prepared organic extracts, 50 μL of $FeSO_4$ (2 mM) was added to a vial containing 1 mL of each extract of *E. velutina* (Ridl.) R. M. Sm in methanol at 2000 μg/mL and 2 mL of distilled water. The reaction was initiated by addition of 100 μL of ferrozine (5 mM). The reaction mixture was then shaken well and incubated at room temperature for 10 min. The absorbance was measured at 562 nm. All the measurements were run in triplicate. Positive controls including EDTA, citric acid (CitA) and AscA were used and the percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following equation:

$$Inhibition\% = [(A_c - A_s)/A_c] \times 100 \qquad \text{(Eqn. 3)}$$

Where A_c is the absorbance of the control composed of 50 μL of the $FeSO_4$, 100 μL of the ferrozine, and 1 mL of methanol, and A_s denotes the absorbance of the extract.

2.4. Antimicrobial activity assays

To evaluate the antimicrobial activity of the organic extracts from different organs of *E. velutina* (Ridl.) R. M. Sm, the corresponding standard methods were taken into consideration with slight modification (Mahdavi et al., 2012), while using two antimicrobial assays, namely disc diffusion and minimum inhibitory concentration (MIC).

2.4.1. Microorganisms

The studied bacterial strains consisted of six Gram-positive strains, namely *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 11774, *Bacillus thuringiensis* ATCC 10792, *Enterococcus faecalis* ATCC 14506, *S. epidermidis* ATCC 12228, and Methicillin Resistant *S. aureus* (MRSA) along with ten Gram-negative strains, namely *Aeromonas hydrophila* ATCC 7966, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 10536, *Proteus vulgaris* ATCC 33420, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhimurium* ATCC 51812, *Serratia marcescens* ATCC 13880, *Shigella sonnei* ATCC 29930, and *Vibrio parahaemolyticus* ATCC 17802. In addition, two *Candida* species, namely *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were used for the evaluation of the corresponding antifungal activity of the prepared organic extracts.

2.4.2. Disc diffusion assay

The microorganism suspensions containing 10^8 CFU/mL were loaded on a sterile cotton swabs and streaked over the dried surface of Mueller-Hinton agar plates for inoculation. The sterile filter paper discs (6 mm in diameter) were impregnated with 20 μL (2 × 10 μL) of each extract (100 mg/mL) and then placed on the inoculated agar. The plates were incubated at 37 °C for 24 h. Antimicrobial activity was determined by measuring the diameter of inhibition zone against the microorganisms. The inhibition zone diameter was measured in millimeters including the disc diameter. All tests were carried out in triplicate. Chloramphenicol (30 μg) and nystatin (30 μg) were used as the positive controls. A disc which was impregnated with 20 μL of solvent was also used as the negative control. For this assay, the extracts of the *E. velutina* (Ridl.) R. M. Sm were dissolved in acetone or methanol.

2.4.3. Minimum inhibitory concentration (MIC)

To determine the MIC values, the 96-well plate (8×12 wells) were first dispensed with 100 μL of the Mueller-Hinton Broth (MHB) culture medium andthe first well was charged with 100 μL of DMSO solution of the extracts. Then, 100 μL from each of their serial dilutions was transferred into consecutive wells, and finally each well charged with 50 μL of the MHB and 50 μL of the bacteria or fungi inoculums which was described earlier. The final volume in each well was 200 μL with concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 mg/mL for each extract and concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 μg/mL for the positive controls (chloramphenicol against the bacteria and nystatin against the fungi). Each plate was only used for one microorganism. Three wells in the last line of the plate were used as growth controls by filling 50 μL of inoculums and 150 μL of MHB. Also, three wells were used as negative control by filling 200 μL of MHB. The covered plates were incubated at 37 °C for 24 hours in an incubator. The turbidity of each well was then observed and recorded. The MIC was assessed as the minimum concentration that resulted in no visible growth. All tests were carried out in triplicate.

2.5. Antiproliferative assay

The cytotoxicity of methanolic extract from different organs of *E. velutina* (Ridl.) R. M. Sm was evaluated on human prostate adenocarcinoma cells (LNCaP cells) using MTT (3-(4,5-dimethylthi-azol-2-yl)-2,5-diphenyltetrazolium bromide) assay according to some previously reports (Ni et al., 2019; Li et al., 2020). In this regard, the cells were evenly distributed in 96-well plates and incubated at 37 °C with 5% CO_2 overnight. In

the next step, the cells were treated with extracts and incubated for 24 h. Then, the medium was replaced with 20 µL MTT (5 mg/mL in FBS) and incubated at 37 °C for 4 h. The formazan crystals were finally dissolved in 100 µL of dimethyl sulfoxide (DMSO) and the absorbance was measured at 510 and 630 nm using a plate reader (Thermo Lab systems, Franklin, MA USA). Finally, IC_{50} as the concentration of the extract totally causing 50% of mortality was calculated.

3. Results and Discussion

3.1. Antioxidant activity

3.1.1. Total phenolic content (TPC)

The total phenolic content of the non-consecutive extracts from the different parts of *E. velutina* (Ridl.) R. M. Sm are shown in Fig. 2.

As seen, for the different plant organs, a decreasing order in each case was noted: for the leaf extracts (ML > AL > EtL > EaL) respectively having the numerical values of 38.34 ± 1.31, 34.11 ± 1.47, 32.04 ± 1.86, 25.82 ± 2.36 mg GAE/g. Here Ea, A, M and Et are respectively referred to ethyl acetate, acetone, methanol and ethanol as the extracting solvents, while R, S and L respectively stand for rhizome, stem and leaf of the plant and *v* represents the plant used (*E. velutina* Ridl. R. M. Sm.). According to the experimental findings of this study, the calculated TPCs for the stem extracts in terms of mg GAE/g were according to the following pattern: MS (25.82 ± 2.36) > AS (22.11 ± 1.61) > EtS (21.63 ± 1.49) > EaS (17.92 ± 1.37). Finally, the TPCs (mg GAE/g) for rhizome extracts had the following order: MR (30.62 ± 0.96) > AR (29.22 ± 1.75) > EtR (26.00 ± 0.40) > EaR (17.16 ± 1.23).

All the extracts from different parts of *E. velutina* (Ridl.) R. M. Sm showed higher TPC values compared to the extracts of the same part of *E. sayapensis* A.D.Poulsen

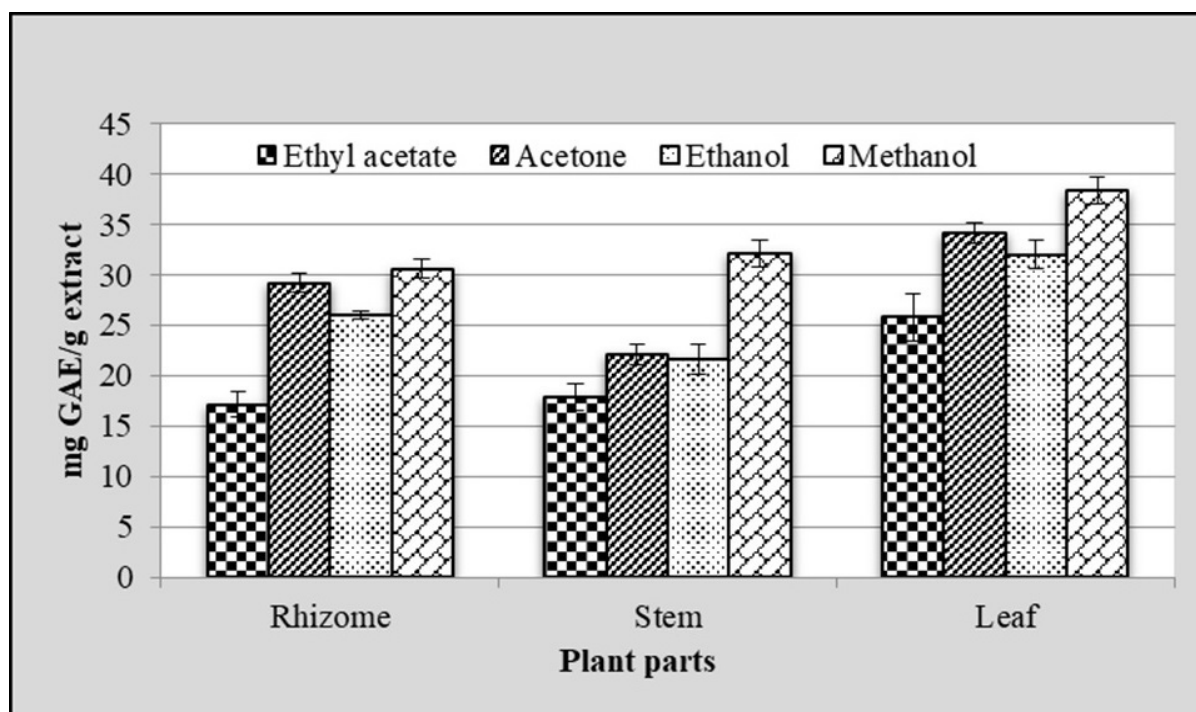


Fig. 2. Representation of total phenolic contents (TPCs) of non-consecutive organic extracts from the different parts of *E. velutina* (Ridl.) R. M. Sm. Values are presented as means ± SD (n = 3).

extracts of the same part of *E. sayapensis* A.D.Poulsen & Ibrahim and *E. brevilabrum* (Valeton) R.M.Sm. Some authors have determined the TPC values for other Malaysian *Etlingera* species growing in different climatic and geographic areas of the country (Chan et al., 2007; Chan et al., 2009). In this context, the TPC values of methanolic extract of the plant leaves collected from Pahang were in a decreasing order: *E. elatior* (Jack) R.M.Sm. > *E. rubrostriata* (Holttum) C.K.Lim > *E. littoralis* (J.Koenig) Giseke > *E. fulgens* (Ridl.) C.K.Lim > *E. maingay* (Baker) R.M.Sm.. On the other hand, the plant leaves collected from Selangor showed higher TPC values for *E. elatior* (Jack) R.M.Sm. followed by *E. rubrostriata* (Holttum) C.K.Lim, *E. littoralis* (J.Koenig) Giseke, and *E. fulgens*. Andarwulan et al. (2010) have reported the TPC of the ethanolic extract of the leaves of *E. elatior* (Jack) R.M.Sm. from Indonesia being lower compared to those of *E. velutina* (Ridl.) R. M. Sm. In addition, in a similar report, Yahya et al. (2011) have implied the TPC of methanolic extracts from different parts of two *Etlingera* species, namely *triogyalis* (Baker) R. M. Sm. and *E. sphaerocephala* var. *grandiflora*. In accordance with this study, a descending order was observed for *E. triogyalis* (Baker) R. M. Sm. as follows: leaf > stem > rhizome with the numerical values of 28.02 ± 1.65, 16.71 ± 1.29, and 4.77 ± 0.66 mg GAE/g, respectively. Furthermore, a similar order was observed for *E. sphaerocephala* var. *grandiflora*: leaf, stem, and

rhizome (33.72 ± 1.98, 14.57 ± 1.27, and 6.72 ± 1.00 mg GAE/g, respectively). From the reported results, it can be concluded that the methanolic extracts from the stems of *E. velutina* (Ridl.) R. M. Sm and *E. sayapensis* A.D.Poulsen & Ibrahim (32.16 ± 1.29, 24.21 ± 1.00 mg GAE/g) was richer than the other *Etlinger* species in TPC values. It is also demonstrated that the TPC values of the leaf extracts of *Etlingera* species are more than those of the stem and rhizome extracts.

3.1.2. Radical scavenging activity (RSA%)

Table 1 shows the RSA (%) results of the non-consecutive extracts from different parts of *E. velutina* (Ridl.) R. M. Sm. Accordingly, the leaf extracts possessed the highest activity but less than all the positive controls. As seen in Table 1, for each plant organ, the lowest and the highest IC_{50} values, were respectively due to methanol and ethyl acetate extracts reflecting the higher antioxidant potentiality of the polar extracts compared to semi-polar nature of the ethyl acetate extracts. The IC_{50} (µg/mL) of the leaf extracts were in an ascending order: ML (58.64 ± 5.31) > AL (206.07 ± 9.01) > EtL (283.11 ± 8.63) > EaL (368.03 ± 8.23) and the IC_{50}s for different extracts of the stem part were found to be respectively as: 141.50 ± 7.69, 420.80 ± 7.63, 589.32 ± 11.17, and 503.21 ± 6.41 µg/mL (MS > AS > EtS > EaS). For the rhizome part, the numerical values of IC_{50} had a similar trend with those of the leaf extracts as: 176.72 ± 6.68, 461.20 ± 6.87, 340.32 ± 9.01, and 642.86 ± 8.03 µg/mL (MR > AR > EtR > EaR). As can be seen for *E. velutina* (Ridl.) R. M. Sm extracts, those obtained using the polar solvent showed higher antioxidant activity.

Previous studies on RSA of the leaves of the other *Etlingera* species were carried out by Chan et al. (2007; 2009). Based on the relevant numerical values of IC_{50}, the species from Pahang were found in a decreasing order of effectiveness as follows. *E. elatior* (Jack) R.M.Sm. > *E. rubrostriata* (Holttum) C.K.Lim > *E. littoralis* (J.Koenig) Giseke > *E. fulgens* (Ridl.) C.K.Lim. The methanolic extracts of the rhizomes from *E. elatior* (Jack) R.M.Sm. and *E. maingayi* exhibited negligible radical scavenging activity regarding their estimated IC_{50} values being 2000 µg/mL (Chan et al. 2008).

Chan et al. (2007) have also determined the BCB activity of several *Etlingera* species. From their presented results, it can be concluded that for the leaf methanolic extracts, the BCB activity was in an order of: *E. maingayi* > *E. elatior* (Jack) R.M.Sm. > *E. rubrostriata* (Holttum) C.K.Lim > *E. littoralis* (J.Koenig) Giseke. The leaf extract of *E. elatior* was more active than the rhizome one. According to previous studies, there was no correlation between DPPH radical scavenging and BCB activity since their reaction mechanisms are different. This may be most probably due to additive, antagonistic or synergistic effects between the components in the complex chemical composition of the plant extracts (Chan et al., 2007; Lim and Quah, 2007).

3.1.3. β-Carotene bleaching (BCB) activity

β-Carotene bleaching (BCB) is considered as a usual assay to measure antioxidant ability of compounds capable of preventing lipid peroxidation. The main components of the BCB assay are β-carotene, linoleic acid, and oxygenated water. The mechanism of bleaching is based on the fading of the yellow color of β-carotene in the presence of linoleic acid free radicals produced when dissolving oxygen in water. Compounds having the potency to inactivate free radicals would inhibit the radical chain reactions resulting in the stability of the β-carotene color in the reaction medium. On the other hand, compounds that are capable of accelerating the free radical formation, would increase the β-carotene bleaching. Hence, this assay is effective when evaluating the pro-oxidative or antioxidative nature of a variety of compounds. Heat and light induced the process through increasing the antioxidative property of the compounds (Wettasinghe and Shahidi, 1997; Duan et al., 2006; Liu, 2010; Moyo et al., 2010). Concerning the *E. velutina* (Ridl.) R. M. Sm extracts, it was observed that the leaf extracts were able to prevent β-carotene bleaching better than the stem and rhizome extracts. Moreover, the methanolic extract of the leaves (ML: 82.36 ± 2.29%) and stems (MS: 80.69 ± 1.32%) were found to be close to each other in terms of their antioxidant activity using the BCB assay. More specifically, these two methanolic extracts retarded bleaching greater than the positive controls of BHT, GA, and AscA. Similar to the *E. sayapensis* A.D.Poulsen & Ibrahim extracts, the BCB ability of *E. velutina* (Ridl.) R. M. Sm extracts exhibited solvent polarity-dependent nature.

3.1.4. Ferrous ion chelating (FIC) assay

Lipid oxidation plays an important role in the oxidative damage of low-density lipoprotein (LDL) and food deterioration (Ayala et al., 2014; Mozuraityte et al., 2016; Kiokias et al., 2018). This process can proceed through radical chain reactions in which the transition metal ions such as iron and copper are the most usual initiators for both biological systems and food materials (Antolovich et al., 2002). Hence, the metal chelating property of the other compounds such as synthetic antioxidants or plant extracts may retard the lipid oxidation *via* complex formation with metal ions. Ferrozine is a well-known reagent to form a stable complex with ferrous ion quantitatively because of its high sensitivity and also low cost. In the presence of other chelating substances, the ferrozine-iron complex disrupts and the complex color (purple) fades (Stookey, 1970; Singh et al., 2010).

The ferrous ion chelating of the non-consecutive extracts from different parts of *E. velutina* (Ridl.) R. M. Sm are tabulated in Table 1. In this relation, the leaf extracts showed the highest FIC ability followed by the stem and rhizome extracts. The methanolic extracts were more potent to chelate ferrous ion compared with the other types of extracts viz. ethanol, acetone, and ethyl acetate for each plant organ and ML*v* with an IC_{50} of 247.24 ± 5.84 µg/mL was determined as the most active plant extract. Considering the findings of this study and similar to the *E. sayapensis* A.D.Poulsen & Ibrahim extracts, all the extracts exhibited a concentration-dependent FIC ability. The positive controls of CitA and AscA were found to have less activity in the FIC assay than the *E. velutina* (Ridl.) R. M. Sm extracts, whereas

Table 1
DPPH radical scavenging activity, β-carotene bleaching, and ferrous ion chelating ability of the non-consecutive extracts of the rhizomes, stems, and leaves of *E. velutina* (Ridl.) R. M. Sm.

Extract	**RSA IC_{50} (μg/mL)**	**BCB (%)**	**FIC (%) IC_{50} (μg/mL)**
Rhizome			
EaR	642.86 ± 8.03d	38.66 ± 3.50d	437.51 ± 9.52d
AR	461.20 ± 6.87c	60.39 ± 2.53c	333.43 ± 7.54c
EtR	340.32 ± 9.01b	64.20 ± 2.25b	329.79 ± 6.99b
MR	176.72 ± 6.68a	76.06 ± 3.42a	284.61 ± 12.79a
Stem			
EaS	503.21 ± 6.41	44.80 ± 3.91d	383.60 ± 5.78d
AS	420.80 ± 7.63	60.51 ± 1.99c	333.37 ± 9.98c
EtS	589.32 ± 11.17	71.57 ± 2.51b	326.43 ± 10.77b
MS	141.50 ± 7.69	80.69 ± 1.32a	282.50 ± 5.42a
Leaf			
EaL	368.03 ± 8.23d	51.02 ± 3.69d	382.49 ± 8.86d
AL	206.07 ± 9.01c	63.55 ± 3.45c	314.79 ± 7.68c
EtL	283.11 ± 8.63b	75.54 ± 1.53b	305.90 ± 7.28b
ML	58.64 ± 5.31a	82.36 ± 2.29a	247.24 ± 5.84a
Standard			
BHT	14.89 ± 0.89b	71.99 ± 4.44	-
GA	7.86 ± 1.23a	44.24 ± 1.33	-
Toc	-	87.90 ± 2.53	-
AscA	40.28 ± 1.06c	21.83 ± 2.04	1426.64 ± 67.43
CitA	-	-	1446.78 ± 88.25
EDTA	-	-	67.23 ± 5.21

*Values are presented as means ± SD (n = 3). Means with different letters are significantly different in each column ($p < 0.05$).

EDTA displayed stronger tendency to chelate ferrous ions.

It has been shown under the experimental conditions that compounds which can form σ-bond(s) with a metal serve as effective metal chelators and known as secondary antioxidants since they can decrease the lipid oxidation *via* stabilizing the oxidized form of the metal ion (Gülçin et al., 2004). The ability of phenols to chelate metal ions in the reaction medium depends on some relevant parameters involving the phenolic structures along with number and position of the hydroxyl groups. However, it has been reported that other compounds such as polysaccharides, phytochelatins and nitrogen-containing compounds are more effective metal chelators than the phenolic compounds and there was no significant correlation between the phenolic content of a plant extract with its metal ion chelating ability (Chan et al., 2007; Wang et al., 2009b). These reports are in agreement with our obtained results by FIC assay on the stem extracts of *E. brevilabrum* (Valeton) R.M.Sm. which despite their lowest TPC, they display high FIC ability comparable to those of other extracts.

3.2. Antimicrobial activity

3.2.1. Disc diffusion assay

The rhizome and stem extracts of *E. velutina* (Ridl.) R. M. Sm were found to be inactive using the disc diffusion antimicrobial assay. However, the leaf extracts gave inhibition against 6 out of 18 tested microorganisms comprising some Gram-positive bacterial strains, *e.g.*, *B. subtilis*, *S. epidermidis* as well as some Gram-negative bacteria, *e.g.*, *E. aerogenes*, *S. sonnei*, *V. paraehaemolyticus*, and a fungus (*C. parapsilosis*) (Table 2). Moreover, ML moderately inhibited *S. sonnei* and *S. epidermidis* strains with inhibition levels of 54.6 and 50.6%. For the other strains and extracts, the inhibition levels were not so remarkable ranging from 28.9 to 47.3%. ML (87.2%) also exhibited the highest antifungal

activity followed by EtL (79.4%), AL (73.4%), and EaL (71.6%). The antimicrobial activity of the extracts considerably depends on the polarity of extracting solvents. Our study also confirmed that ethyl acetate and methanol extracts were categorized as giving the weakest and strongest antimicrobial activity.

3.2.2. Minimum inhibitory concentration results

The MIC results of the leaf extracts of *E. velutina* (Ridl.) R. M. Sm were tabulated in Table 3. As the obtained results in this table show, ML had the highest bacteriostatic activity against *E. aerogenes*, *B. subtilis*, *V. paraehaemolyticus*, *S. sonnei*, and *S. epidermidis* with MIC values of 1.56 ± 0.00, 2.34 ± 1.35, 2.60 ± 0.90, 3.12 ± 0.00, 8.33 ± 3.60 mg/mL, respectively. Additionally, ML also retarded the growth of *C. parapsilosis* with the lowest MIC (2.60 ± 0.90 mg/mL). However, the antimcrbial activity of the extracts from different parts of *E. velutina* (Ridl.) R. M. Sm was less than the positive controls (chloramphenicol or nystatin). It has been well documented that antimicrobial activity of the individual compounds depends on some experimental factors such as absorbability, electron density, molecular hydrophobicity, and surface activity (Park et al., 2001). This feature for the plant extracts is also dependent on the extraction method, compounds class and quantity of secondary metabolites of the studied extracts. The antimicrobial activity is also associated with the culture medium and growth phase of cultivation used for analysis (Shaaban et al., 2012). The plant-origin extracts possess the antimicrobial activity because of having phenolic compounds and other secondary metabolites, *e.g.*, tannins, flavonoids, terpenoids, and alkaloids (Kil et al., 2009). In addition, the synergistic effect of the different chemical constituents of the plant extracts is known as another principle reason of the antimicrobial effects of the plant extracts (Amanlou et al., 2004). It can be concluded that the antimicrobial activity of the extracts from different parts of *Etlingera* species was related to the presence of various classes of natural compounds in their characterized profiles.

A simple comparison of the antimicrobial activity reported for the other *Etlingera* species resembles that the leaf methanolic extracts of *E. elatior* (Jack) R.M.Sm., *E. fulgens* (Ridl.) C.K.Lim, *E. maingayi* (Baker) R.M.Sm., *E. rubrostriata* (Holttum) C.K.Lim, and *E. littoralis* (J.Koenig) Giseke moderately prevented the growth of *S. aureus* strain (Chan et al. 2007). Yahya (2011) also reported the moderate ability of methanolic extracts of the rhizomes, stems, and leaves of *E. sphaerocephala* var. *grandiflora* and *E. triorgyalis* (Baker) R.M.Sm. to inhibit the growth of *S. aureus*.

3.3. Anti-prostate cancer activity

Antiproliferative assay is one of the widely used and practical approaches to evaluate the bioactivity of the natural products representing an extract, essential oil, or compound's capability to reduce the growth of a given cell population (Ni et al., 2019; Al-Mansoub et al., 2021). Fig. 3 shows the results of the antiproliferative activity of methanolic extract from the different parts of *E. velutina* (Ridl.) R. M. Sm. The findings expressed that the extracts exhibited a dose-related activity on the selected cell lines. For ML, the highest activity was obtained for LNCaP (IC_{50} = 110.97 ± 2.65 µg/mL) followed by MS (IC_{50} = 132.99 ± 3.60 µg/mL) and MR (IC_{50} = 171.44 ± 1.98 µg/mL). A literature survey demonstrates that there have been some sporadic reports on potential anticancer potentials of some *Etlingera* extracts. In this sense, *E. elatior* (Jack) R.M.Sm. extract has decreased the viability of immortal and breast cancer cells (Ghasemzadeh et al., 2015; Al-Mansoub et al., 2021). Furthermore, the extract of *E. pubescens* (B.L.Burtt & R.M.Sm.) R.M.Sm. has been found active against human colon cancer cells (Daniel-Jambun et al., 2018) and *E. pavieana* promoted the survival of breast and cervical cancer cells (Iawsipo et al., 2018).

E. elatior (Jack) R.M.Sm. extract has decreased the viability of skin and colon cancer cells (Ghasemzadeh et al., 2015; Al-Mansoub et al., 2021). The extract of *E. pubescens* (B.L.Burtt & R.M.Sm.) R.M.Sm. has been found active against human colon cancer cells (Daniel-Jambun et al., 2018). *E. pavieana* promoted the survival of breast and cervical cancer cells (Iawsipo et al., 2018).

4. Concluding remarks

Emerging new and unknown types of persistent diseases arising from a number of free radicals along with bacterial or microbial strains have encouraged scientists to focus on remarkable natural antioxidant and antibacterial agents with plant origin. This research topic has gained much attention within the past few decades giving rise in number of natural alternatives capable of scavenging free radicals or inhibiting different types of microbes, *e.g.*, bacteria and fungi. Furthermore, the evaluation of anticancer activity of plants extracts as the potent agents with minimum side effects is favorable filed in phytochemi-cal-based studies. In the present research, we have reported the antioxidant, antimicrobial, and anticancer activity of extracts from different parts of *velutina* (Ridl.) R. M. Sm . The results obtained revealed that the plant can be considered as a strong agent for the selected biological evaluatioins.

Abbreviations

AscA: Ascorbic Acid; **AL:** Acetone Leaf; **AR:** Acetone Rhizome; **AS:** Acetone Stem; **α-Toc.:** α-Tocopherol; **BCB:** β-Carotene Bleaching Activity; **BHT:** Butylated Hydroxytoluene; **CitA:** Citric Acid; **DMSO:** Dimethyl Sulfoxide; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EaL:** Ethyl Acetate Leaf; **EaR:** Ethyl Acetate Rhizome; **EaS:** Ethyl Acetate Stem; **EDTA:** Ethylenediaminetetraacetic Acid; **EtL:** Ethanol Leaf; **EtR:** Ethanol Rhizome; **EtS:** Ethanol Stem; **FBS:** Fetal Bovine Serum; **FIC:** Ferrous Ion Chelating; **FCR:** Folin-Ciocalteu Reagent; **GA:** Gallic Acid; **MHB:** Mueller-Hinton Broth; **MIC:** Minimum Inhibitory Concentration; **ML:** Methanol Leaf; **MR:** Methanol Rhizome; **MRSA:** Methicillin Resistant *S. aureus*; **MS:** Methanol Stem; **MTT:** 3-(4,5-Dimethylthi-azol-2-yl)-2,5-Diphenyltetrazolium Bromide; **RSA:** Radical

Table 2
Antibacterial activity of the leaf extracts of *E. velutina* (Ridl.) R. M. Sm using disc-diffusion assay[a].

Bacterial strain type	Inhibition zone diameter (mm) and inhibition level (%)						
Gram-positive		**EaL**	**AL**	**EtL**	**ML**	**Positive Control[b]**	**Negative Control[c]**
B. subtilis	mm	6.0[d] ± 0.0	6.0 ± 0.0	10.2 ± 0.8[e]	9.6 ± 0.6	28.9 ± 0.8	6.0 ± 0.0
	%	-	-	33.2	35.3	-	-
S. epidermidis	mm	8.1 ± 0.8	9.4 ± 0.5	11.5 ± 0.5	13.8 ± 0.8	27.2 ± 0.6	6.0 ± 0.0
	%	29.8	34.6	42.3	50.7	-	
Gram-negative							
E. aerogenes	mm	9.2 ± 0.6	11.2 ± 0.3	11.6 ± 0.4	12.3 ± 0.5	26.0 ± 0.6	6.0 ± 0.0
	%	35.4	43	44.6	47.3		-
S. sonnei	mm	9.2 ± 0.3	10.5 ± 0.5	10.6 ± 0.6	13.6 ± 0.6	24.9 ± 0.3	6.0 ± 0.0
	%	36.9	42.2	42.6	54.6		-
V. parahaemolyticus	mm	8.3 ± 0.8	9.3 ± 0.5	9.9 ± 0.6	11.6 ± 0.4	28.7 ± 0.6	6.0 ± 0.0
	%	28.9	34.5	32.4	40.4		-
Fungi							
C. parapsilosis	mm	10.1 ± 0.7	10.3 ± 0.5	11.2 ± 0.6	12.3 ± 0.5	14.1 ± 0.4	6.0 ± 0.0
	%	71.6	73	79.4	87.2	-	-

[a]Each disc was impregnated with 20 µL of extract at 100 mg/mL; [b]Chloramphenicol 30 µg and nystatin 30 µg for fungal; [c] Solvent; [d]size of discs (6 mm), non-active; [e]Values are presented as means ± SD (*n* = 3).

Table 3
Minimum inhibition concentration (MIC) of the leaf extracts of *E. velutina* (Ridl.) R. M. Sm.

Gram-positive	**EaL (mg/mL)**	**AL (mg/mL)**	**EtL (mg/mL)**	**ML (mg/mL)**	**Control[a] (mg/mL)**
B. subtilis	-	-	2.60 ± 0.90[b]	2.34 ± 1.35	0.02 ± 0.01
S. epidermidis	12.50 ± 0.00	10.42 ± 3.61	10.42 ± 3.61	8.33 ± 3.60	0.01 ± 0.00
Gram-negative					
E. aerogenes	3.12 ± 0.00	2.60 ± 0.90	2.34 ± 1.35	1.56 ± 0.00	< 0.01
S. sonnei	4.16 ± 1.81	5.29 ± 1.88	4.16 ± 1.81	3.12 ± 0.00	< 0.01
V. parahaemolyticus	6.25 ± 0.00	4.16 ± 1.81	3.12 ± 0.00	2.60 ± 0.90	< 0.01
Fungi					
C. parapsilosis	4.16 ± 1.81	4.16 ± 1.81	3.12 ± 0.00	2.60 ± 0.90	0.02 ± 0.01

[a]Chloramphenicol and nystatin for fungal; [b]Values are presented as means ± SD (*n* = 3).

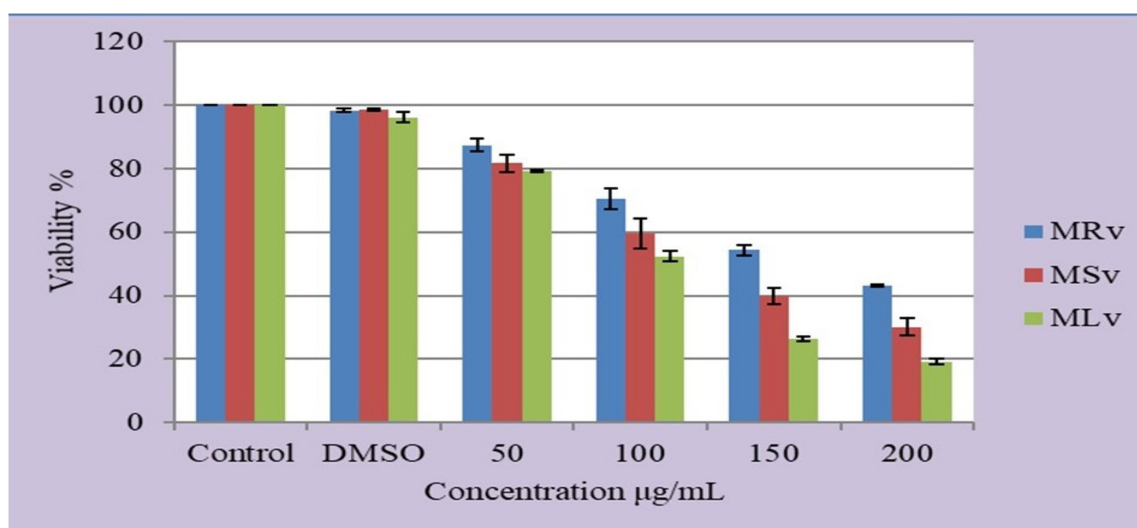


Fig. 3. Representation of antiproliferative activity of methanolic extract of different parts of *E. velutina* (Ridl.) R. M. Sm.

Scavenging Activity; **TPC:** Total Phenolic Content.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgements

Financial and technical supports of Department of Chemistry, Faculty of Science, Hakim Sabzevari University, Sabzevar, Iran as well as Islamic Azad University of Shahrood are gratefully acknowledged.

References

A.M. Abdel-Rahman, I., E. Raslan, A., Dawy, A., Wail, A., Ahmed, F., Hany, K., M. Al-Monair, M., Ahmed, O., I. Rushdi, M., A.E. Allam, A., 2022. *Cymbopogon schoenanthus* (L.) Spreng: A Comprehensive review on phytochemical and pharmacological biodiversity Trends Phytochem. Res. 6(4), 259-281.

Al-Mansoub, M.A., Asif, M., Revadigar, V., Hammad, M.A., Chear, N.J.Y., Hamdan, M.R., Majid, A.M.S.A., Asmawi, M.Z., Murugaiyah, V., 2021. Chemical composition, antiproliferative and antioxidant attributes of ethanolic extract of resinous sediment from *Etlingera eatior* (Jack.) inflorescence. Bras. J. Pharm. Sci. 57.

Amanlou, M., Fazeli, M.R., Arvin, A., Amin, H.G., Farsam, H., 2004. Antimicrobial activity of crude methanolic extract of *Satureja khuzistanica*. Fitoterapia 75(7-8), 768-770.

Andarwulan, N., Batari, R., Sandrasari, D.A., Bolling, B., Wijaya, H., 2010. Flavonoid content and antioxidant activity of vegetables from Indonesia. Food Chem. 121(4), 1231-1235.

Antolovich, M., Prenzler, P.D., Patsalides, E., McDonald, S., Robards, K., 2002. Methods for testing antioxidant activity. Analyst 127(1), 183-198.

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 2014.

Chan, E., Lim, Y., Omar, M., 2007. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. Food Chem. 104(4), 1586-1593.

Chan, E.W.C., Lim, Y.Y., Ling, S.K., Tan, S.P., Lim, K.K., Khoo, M.G.H., 2009. Caffeoylquinic acids from leaves of *Etlingera* species (Zingiberaceae). LWT Food Sci. Technol. 42(5), 1026-1030.

Christenhusz, M.J., Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa 261(3), 201–217-201–217.

Colegate, S.M., Molyneux, R.J., (Eds) 2008. Bioactive Natural Products Detection, Isolation, and Structural Determination. Vol. 1, 2nd Ed., CRC Press,Taylor & Francis Group, New York.

Daniel-Jambun, D., Ong, K.S., Lim, Y.Y., Lee Tan, J.B., Lee, W.L., Muhamad, A., Yap, S.W., Lee, S.M., 2018. Antioxidant properties of *Etlingera pubescens*, an edible ginger plant endemic to Borneo. Food BioSci. 25, 44-51.

Duan, X.-J., Zhang, W.-W., Li, X.-M., Wang, B.-G., 2006. Evaluation of antioxidant property of extract and fractions obtained from a red alga, *Polysiphonia urceolata*. Food Chem. 95(1), 37-43.

Ghasemzadeh, A., Jaafar, H.Z.E., Rahmat, A., Ashkani, S., 2015. Secondary metabolites constituents and antioxidant, anticancer and antibacterial activities of *Etlingera eatior* (Jack) R.M.Sm grown in different locations of Malaysia. BMC Complement. Altern. Med. 15(1), doi: 10.1186/s12906-015-0838-6.

Gülçin, Ì., Güngör Sat, I., Beydemir, S., Elmastas, M., Irfan Küfrevioglu, Ö., 2004. Comparison of antioxidant activity of clove (*Eugenia caryophylata Thunb)* buds and lavender (*Lavandula stoechas* L.). Food Chem. 87(3), 393-400.

Harvey, A., 2000. Strategies for discovering drugs from previously unexplored natural products. Drug Discov. Today 5(7), 294-300.

Iawsipo, P., Srisook, E., Ponglikitmongkol, M., Somwang, T., Singaed, O., 2018. Cytotoxic effects of *Etlingera pavieana* rhizome on various cancer cells and identification of a potential anti-tumor component. J. Food Biochem. 42(4), doi: org/10.1111/jfbc.12540.

Kazeminia, M., Mehrabi, A., Mahmoudi, R., 2022. Chemical composition, biological activities, and nutritional application of Asteraceae family herbs: A systematic review. Trends Phytochem. Res. 6(3), 187-213.

Kil, H.Y., Seong, E.S., Ghimire, B.K., Chung, I.-M., Kwon, S.S., Goh, E.J., Heo, K., Kim, M.J., Lim, J.D., Lee, D., Yu, C.Y., 2009. Antioxidant and antimicrobial activities of crude sorghum extract. Food Chem. 115(4), 1234-1239.

Kiokias, S., Proestos, C., Oreopoulou, V., 2018. Effect of natural food antioxidants against LDL and DNA oxidative changes. Antioxidants 7(10), 133.

Leland, J.C., Ara, K., Peter B., K., Sara L., W., James A., D., Harry L., B., 2006. Natural Products from Plants. 2nd Ed., CRC Press, Taylor & Francis Group, New York.

Li, M., Mahdavi, B., Yaacob Wan, A., Din Laily, B., 2020. Chemical composition, antioxidant, antimicrobial and anti proliferative activities of essential oil and extract of the fruits of *Etlinger sayapensis*. J. Essent. Oil-Bear. Plant. 23(5), 931-943.

Lim, Y.Y., Quah, E.P.L., 2007. Antioxidant properties of different cultivars of *Portulaca oleracea*. Food Chem. 103(3), 734-740.

Liu, Z.-Q., 2010. Chemical methods to evaluate antioxidant ability. Chem. Rev. 110(10), 5675-5691.

Mahdavi, B., Yaacob, W.A., Laily, B.D., Jahangirian, H., 2013. Antioxidant activity of consecutive extracts of the base, stem and leaves of *Etlingera brevilabrum*. Asian J. Chem. 25(7), 3937-3941.

Mahdavi, B., Yaacob, W.A., Laily, B.D., Nazlina, I., 2012. Antimicrobial activity of consecutive extracts of *Etlingera brevilabrum*. Sains Malays. 41(10), 1233-1237.

Moyo, M., Ndhlala, A.R., Finnie, J.F., Van Staden, J., 2010. Phenolic composition, antioxidant and acetylcholinesterase inhibitory activities of *Sclerocarya birrea* and *Harpephyllum caffrum* (Anacardiaceae) extracts. Food Chem. 123(1), 69-76.

Mozaffarian, V., 2003. Indentification of Medicinal and Aromatic Plants of Iran. Farhang Moaser Press, Tehran, Iran.

Mozuraityte, R., Kristinova, V., Rustad, T., 2016. Encyclopedia of Food and Health. Academic Press Oxford, UK.

Nalawade, A.S., Gurav, R.V., Patil, A.R., Patwekar, M., Patwekar, F., 2022. A comprehensive review on morphological, genetic and phytochemical diversity, breeding and bioprospecting studies of genus *Chlorophytum* Ker Gawl. from India. Trends Phytochem. Res. 6(1), 19-45.

Ni, J., Mahdavi, B., Ghezi, S., 2019. Chemical composition, antimicrobial, hemolytic, and antiproliferative activity of essential oils from *Ephedra intermedia* Schrenk & Mey. J. Essent. Oil-Bear. Plant. 22(6), 1562-1570.

Pardeshi, P.U., Kapile, C.R., Kulkarni, A.D., Gulecha, V.S., Zalte, A.G., Gedam, S.S., 2022. Phytochemical-based

vesicular system for the treatment of vitiligo: A review. Trends Phytochem. Res. 6(3), 224-241.

Park, E.S., Moon, W.S., Song, M.J., Kim, M.N., Chung, K.H., Yoon, J.S., 2001. Antimicrobial activity of phenol and benzoic acid derivatives. Int. Biodeterior. Biodegrad. 47(4), 209-214.

Phillipson, J.D., 2001. Phytochemistry and medicinal plants. Phytochemistry 56(3), 237-243.

Poulsen, A.D., 2006. Etlingera of Borneo, 1st Ed. Natural History Publications (Borneo) in association with Royal Botanic Garden Edinburgh.

Shaaban, H.A.E., El-Ghorab, A.H., Shibamoto, T., 2012. Bioactivity of essential oils and their volatile aroma components: Review. J. Essent. Oil Res. 24(2), 203-212.

Singh, G., Kapoor, I.P.S., Singh, P., de Heluani, C.S., de Lampasona, M.P., Catalan, C.A.N., 2010. Comparative study of chemical composition and antioxidant activity of fresh and dry rhizomes of turmeric (*Curcuma longa* Linn.). Food Chem. Toxicol. 48(4), 1026-1031.

Stookey, L.L., 1970. Ferrozine-a new spectrophotometric reagent for iron. Anal. Chem. 42(7), 779-781.

Wang, T., Jónsdóttir, R., Ólafsdóttir, G., 2009b. Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. Food Chem. 116(1), 240-248.

Wettasinghe, M., Shahidi, F., 1997. Antioxidant activity of preformed cooked cured-meat pigment in a β-carotene/linoleate model system. Food Chem. 58(3), 203-207.

WorldBank, 2004. Sustaining Forest. University, Washington, D.C.

Yahya, M.A.A., Yaacob, W.A., Nazlina, I., 2011. Isolation of chemical constituents from rhizomes of *Etlingera sphaerocephala* var. *grandiflora*. Malays. J. Anal. Sci. 15(1), 22-26.

Zargari, A., 1991. Medicinal Plants Tehran University Press, Tehran, Iran.